

## Age-stratified Sero-prevalence and Risk factors of Human Papillomavirus (HPV): Implications for Vaccination in Nigeria

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**Abstract:** Human papillomavirus (HPV) is a naked, double-stranded DNA virus that is often responsible for benign lesions of the skin and mucous membranes. Certain strains of HPV have also been implicated in the development of epithelial malignancies. In this study, we investigated age prevalence of IgG antibodies to human papillomavirus16 and 18 among patients of age 1-65 years attending Family Medicine Department of the University of Ilorin Teaching Hospital, Ilorin using ELISA techniques. Out of the 174 consented participants, the prevalence of IgG antibodies to HPV, HPV16 and HPV18 in this study is 3.4%, 0.6% and 1.7% respectively. A seroprevalence of 7.1% in the under 5 age-group was found in this cross-sectional study. The correlation between the risk factors and HPV seroprevalence were however not statistically significant. The prevalence of IgG antibody observed also shows that majority of the subjects are still at risk of HPV infection and this highlights the potential roles of massive vaccination to provide herd immunity. Seroprevalence among under-5 class calls for inclusion of HPV vaccination among this age group as against current older vaccination age.

**Keywords:** Human Papillomavirus, IgG antibodies, Age, Prevalence, Vaccine

### INTRODUCTION

The Human Papillomavirus (HPV) is a double stranded DNA virus which belongs to the family *Papillomaviridae* containing over 240 genotypes. HPV has been implicated as the cause of some neoplasms including cervical cancer and majority of all cervical cancer cases has been attributed to genital infection with human papillomavirus (HPV) (Van Doorslaer, 2013; WHO, 2019). Cervical cancer is estimated to affect approximately 586,000 women each year with 326,000 deaths, 80% of whom live in developing countries (Ferlay *et al.*, 2010). HPV can be contracted through; sexual intercourse, contaminated shared objects, towels underwear, blood and prenatally (Jajaprakash *et al.*, 2011; Sabeena *et al.*, 2017). The predisposing factors of HPV include number of sexual partners (more than one), age (common warts occur mostly in children and adolescents, while genital warts occur often in adolescents and young adults), immunosuppression, damaged skin, personal contact or unprotected exposure to HPV-contaminated surface (Cutts *et al.*, 2007). The spectrum of diseases associated with HPV has been used to place the virus

into two categories which are low risk and high risk HPVs. Low risk HPVs are types; 1, 2, 3, 4, 6, 7, 8, 10, 11, 42, 44 and 53. They are associated with warts at different anatomical sites of human body but do not cause cancer. On the other hand, high risk HPV types which includes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are associated with a pre-cancerous lesion known as Cervical Intraepithelial Neoplasia (CIN) 2/3 and which may eventually progress to cancer (Cutts *et al.*, 2007).

HPV can be prevented by effective immunization. In the developed countries, well-organized programmes of regular gynaecological screening and treatment of precancerous lesions have helped tremendously in preventing squamous cervical cancer. However, this is not the case in low-resource settings or countries which mostly lack such initiatives (Chew *et al.*, 2005). There is high rate of morbidity and mortality from cervical cancer in the developing countries (Clifford & Franceschi, 2008; CDC, 2013; Nweke *et al.*, 2018). In Nigeria, about 10,000 cases of cervical cancer are reported annually with over 8,000 deaths (Bisi-Onyemaechi, 2018).

Similarly, the prevalence of HPV (16 or 18) among women with cervical cancer is approximately 70%. This figure shows a link between HPV and cases of cervical cancer in the country (Bruni *et al.*, 2017). A recent review on the burden of HPV in Nigeria showed the prevalent types to be 16, 18, 31, 33, 35, 52, 56, and 68 (Omoare *et al.*, 2016). Several methods including Polymerase Chain Reaction (PCR) as well as Enzyme-Linked Immunosorbent Assay (ELISA) have been employed to screen for the activities of HPV in Nigeria among different cohorts with prevalence ranging from 4-76% (Auwal *et al.*, 2013; de Sanjose *et al.*, 2007; Fadahunsi *et al.*, 2013; Paul *et al.*, 2002; WHO, 2010). However, virtually all of these studies focused on adolescent and sexually active women aged between 15 and 45 years. A more comprehensive study that will include pre-adolescent individuals and children in HPV screening is lacking.

Therefore, this study seeks to determine the age stratified prevalence of IgG, a marker of previous exposure, antibodies to HPV, its subtypes 16 and 18, and the associated risk factors among patients attending University of Ilorin Teaching Hospital and Implications for immunization practice in Nigeria.

## MATERIALS AND METHODS

This study is a descriptive cross-sectional research conducted at the University of Ilorin Teaching Hospital, Ilorin, Nigeria. University of Ilorin Teaching hospital (UIITH) caters for patients from North-central and South-western Nigeria. The General Outpatient (GOP) Clinic of the Family Medicine Department is the first contact point for all out-patients in the institution. Using convenience sampling technique, a total of 200 consecutive consenting patients from pre-adolescent age groups to 65 years attending GOP clinic were recruited. Consents for children were obtained from their parents. Of the 200, 174 participants met the inclusion criteria. Subjects who were immunocompromised,

malnourished children, HIV-positive patients, patients on chemotherapy, immunosuppressant, and steroids were excluded. Patients who had already received vaccine for HPV and those who refused to give informed consent were also excluded. Ethical approval (ERC PAN/2013/10/1251) was obtained for this study from Health Research Ethics Committee (HREC) of UIITH (HREC/02/05/2010). Patients' socio-demographic data and information on risk factors were obtained using proforma designed by the authors.

**Sample collection:** 3-5mL of blood sample was aseptically collected into plain bottles from each participant by a trained phlebotomist. Collected blood samples were immediately transported in cold chain to the Microbiology Laboratory for serum separation. Samples on reaching the laboratory were allowed to clot and centrifuged at 12,500rpm for 10minutes. The sera were carefully separated and kept inside cryovials and refrigerated at temperature of -20°C prior to analysis.

ELISA procedure was carried out according to the manufacturer's instruction described by Diapro Diagnostics®, Italy. Samples were diluted using the sample diluent at ratio 1:101 i.e. 100µL sample diluted + 1µL sample. This was mixed on vortex. Negative and Positive controls (100µL each) were dispensed in duplicates into the microwells leaving A1 well empty for the operation of blanking. Diluted samples (100µL) were then dispensed into each properly identified well. They were incubated for 60min at 37°C. The microplates were washed using automatic wash. Enzyme conjugate (100µL) was then added into all the wells except A1 well and then covered with the sealer. Samples were again incubated for 60 min at 37°C. The microwells were thereafter washed. This was followed by addition of 100µL of sulphuric acid into all the wells. The colour intensity of the solution in each well was measured at 450nm filter reading.

Cut-off was the addition of Mean of optical density (OD) of 450nm value of Negative Control (NC) with 0.25. [Cut-off = Mean OD<sub>450nm</sub> of NC + 0.25]. Samples with an optical density lower than the cut-off value were considered negative, while the samples with higher optical density than the cut-off were positive for IgG-specific to the HPV antigen.

Samples tested positive were further tested for HPV 16 and HPV 18 IgG antibody following the manufacturer's procedure. Samples were numbered to correspond with the micro-titration well. Approximately 50µL of positive and negative controls were added to the positive and negative wells respectively. Sample diluent (40µL) was added to testing sample wells followed by addition of 10µL of testing samples and then mixed. After closing plate with closure plate membrane, it was incubated for 30 minutes at 37°C. Following incubation, the plate was uncovered, liquid was discarded, dried by swing and 30-fold diluted-wash solution was added to every well and drained after 30s. This was repeated 5 times and then dried by pat. HRP-conjugate reagent (50µL) was added to each well except the blank well. Samples were then incubated for 30 min at 37°C and thereafter washed with the buffer. Chromogen solution A & B (50µL) was added to the wells. Thereafter, stop solution (50µL) was also added to stop the reaction. Then blank well was taken as zero, absorbance was read at 450nm within 15min after adding the stop solution. Cut-off value was calculated as average of Negative control well + 0.15. Samples with an optical density lower than the cut-off value were

considered negative. While the samples with higher optical density than the cut-off were positive for IgG specific to the HPV16 antigen. The same procedure was repeated to check for HPV 18 IgG.

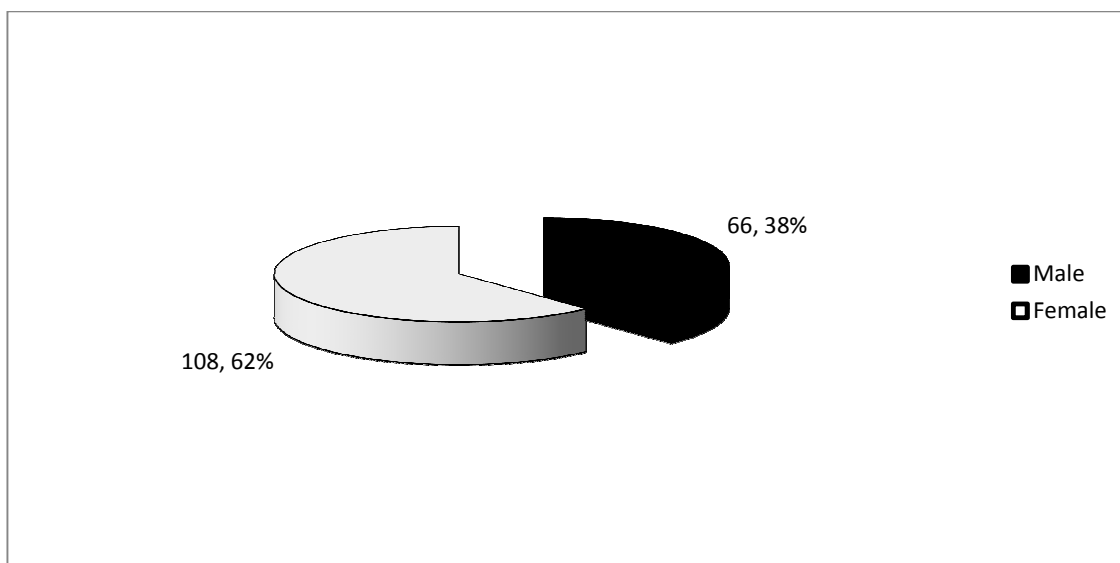
Complete data were collected, sorted and analyzed for 174 participants who met the inclusion criteria. Significant level was set at  $P \leq 0.05$  and categorical data were compared using Chi-square( $\chi^2$ ), while continuous variables were described by means and mode. Data analysis was done using the SPSS Statistics version 20.0 (IBM® Chicago, Illinois, USA).

## RESULTS

Age range was from 1 to 65 with a mean age of 34.6 (standard deviation, SD±19.4). Sixty-six (37.9%) were males while 108 (62.1%) were females (Figure 1).

### Stratified Age Seroprevalence of HPV, HPV-16 and HPV-18

Consequently, six participants (3.45%) were sero-positive for HPV. Only 1 (0.6%) and 3 (1.76%) were HPV-16 and HPV-18 IgG specific respectively. The HPV seroprevalence by their age classes were 7.1% (1-5yrs), 21.4% (36-40yrs) and 15.4% (41-45yrs). HPV antibodies were not found in other age classes. HPV-16 seroprevalence for age class 36-40 was 7.1%. However other age classes have 0% seroprevalence for HPV-16. This study shows HPV-18 seroprevalence of 7.1% and 15.38% were found only in 36-40 and 41-45 age classes respectively (Table 1).



**Figure 1: Gender distribution of participants for HPV, HPV-16 and HPV-18 screening**

**Table 1: Seroprevalence of HPV, HPV16 and HPV18 among the subjects**

| Age (years)  | N          | Prevalence of HPV IgG (%) | Prevalence of HPV 16 IgG (%) | Prevalence of HPV 18 IgG (%) |
|--------------|------------|---------------------------|------------------------------|------------------------------|
| 1-5          | 14         | 1(7.1)                    | -                            | -                            |
| 6-10         | 13         | -                         | -                            | -                            |
| 11-15        | 12         | -                         | -                            | -                            |
| 16-20        | 11         | -                         | -                            | -                            |
| 21-25        | 9          | -                         | -                            | -                            |
| 26-30        | 14         | -                         | -                            | -                            |
| 31-35        | 12         | -                         | -                            | -                            |
| 36-40        | 14         | 3(21.4)                   | 1(7.1)                       | 1(7.1)                       |
| 41-45        | 13         | 2(15.4)                   | -                            | 2(15.38)                     |
| 46-50        | 18         | -                         | -                            | -                            |
| 51-55        | 16         | -                         | -                            | -                            |
| 56-60        | 14         | -                         | -                            | -                            |
| 61-65        | 14         | -                         | -                            | -                            |
| <b>Total</b> | <b>174</b> | <b>6(3.4)</b>             | <b>1(0.6)</b>                | <b>3(1.7)</b>                |

#### **Correlation of Risk Factors with HPV IgG, HPV16 IgG and HPV18 IgG Seroprevalence**

Risk factors considered in this study are history of blood transfusion, multiple sexual partners, sharing of sharp objects, gender, leg laceration, smoking/alcohol consumption, sharing of towel, pedicure and manicure. There was no statistically significant correlation between HPV IgG antibodies prevalence and the risk factors

considered in this study. Among the HPV sero-positive participants; only 17% had been previously transfused, none had multiple sexual partners, 50% admitted to sharing sharp like blade or clipper with people. They had no history of leg laceration, smoking or alcohol consumption, however, 16.7% of HPV positive participants share towel with people while none of them did pedicure or manicure (Table 2).

**Table 2: Correlation of Risk Factors with HPV IgG, HPV16 IgG and HPV18 IgG Seroprevalence**

|                           |            | HPV IgG (%) | HPV 16 IgG | HPV18 IgG |
|---------------------------|------------|-------------|------------|-----------|
| Blood Transfusion         | Yes        | 1 (16.7)    | 0(0.0)     | 0(0.0)    |
|                           | No         | 5(83.3)     | 1(16.7)    | 2(33.3)   |
|                           | Chi square | 0.015       | 0.177      | 0.355     |
|                           | P-value    | 0.904       | 0.674      | 0.551     |
| Number of Sexual partners | None       | 2(33.3)     | 0(0)       | 1(16.7)   |
|                           | Single     | 4(66.7)     | 1(16.7)    | 2(33.3)   |
|                           | Multiple   | 0(0.0)      | 0(0.0)     | 0(0.0)    |
|                           | Chi square | 0.497       | 0.557      | 0.274     |
|                           | P-value    | 0.787       | 0.757      | 0.703     |
| Sharing of sharp objects  | Yes        | 3(50.0)     | 1(16.7)    | 3(50.0)   |
|                           | No         | 3(50.0)     | 0(0.0)     | 0(0.0)    |
|                           | Chi square | 2.009       | 2.972      | 2.767     |
|                           | P-value    | 0.156       | 0.850      | 0.960     |
| Gender                    | Male       | 3(50.0)     | 1(16.7)    | 1(16.7)   |
|                           | Female     | 3(50.0)     | 0(0.0)     | 2(33.3)   |
|                           | Chi square | 0.384       | 1.646      | 0.270     |
|                           | P-value    | 0.53        | 0.200      | 0.869     |
| Leg Laceration            | Yes        | 0(0.0)      | 0(0.0)     | 0(0.0)    |
|                           | No         | 6(100)      | 1(16.7)    | 3(50.0)   |
|                           | Chi square | 0.036       | 0.006      | 0.018     |
|                           | P-value    | 0.850       | 0.939      | 0.894     |
| Alcohol/Smoking           | Yes        | 0(0.0)      | 0(0.0)     | 0(0.0)    |
|                           | No         | 6(100.0)    | 1(16.7)    | 3(50)     |
|                           | Chi square | 0.260       | 0.042      | 0.128     |
|                           | P-value    | 0.610       | 0.837      | 0.721     |
| Sharing of Towel          | Yes        | 1(16.7)     | 0(0.0)     | 1(16.7)   |
|                           | No         | 5(83.3)     | 1(16.7)    | 2(33.3)   |
|                           | Chi square | 0.189       | 0.320      | 0.140     |
|                           | P-value    | 0.660       | 0.570      | 0.707     |
| Pedicure/Manicure         | Yes        | 0(0.0)      | 0(0.0)     | 0(0.0)    |
|                           | No         | 6(100)      | 1(16.7)    | 3(50.0)   |
|                           | Chi-square | 0.397       | 0.730      | 0.553     |
|                           | P-value    | 0.156       | 0.850      | 0.960     |

**DISCUSSION**

Various HPV seroprevalence studies in Nigeria have been carried out but have not underscored early age detection as emphasis has been on the sexually active age groups. This study looked at age 1 to 65. On overall view, the prevalence seen in this study (3.4%) is similar to what Oboma & Avwioro (2012) found in Bayelsa, South-south region of Nigeria (4.2%). Several studies have shown that HPV 16 and 18 are the most

prevalent among the high-risk HPV (Newall *et al.*, 2008; Okunade *et al.*, 2017). Studies have also indicated that some genotypes cross react with HPV16 and 18, which accounts for their cross protection by the vaccine preventable HPV types. Nonetheless, other high-risk groups were reported to be prevalent that are not cross reacting with HPV 16 and 18 (Bruni *et al.*, 2010; CDC, 2010).

Our study revealed two positive sera that obviously did not cross-react with 16 and 18, with implication that individual whose genotypes are out of the ones for which vaccines had been manufactured may not be protected, in case their infections progressed to the stage of development of Cervical Intraepithelial Neoplasia (CIN) 2/3 which are pre-cancerous lesions.

Although 7.1% of the individuals in age range 1-5 years were positive for HPV 16, we were not able to type the HPV to which some cases have been exposed to because our study only detected previous exposures to HPV 16 and 18. This study reported peak prevalence for HPV 16 (7.1%) and HPV 18 (15.4%) among age 36-45 similar to other studies like Newall *et al.* (2008) whose study revealed 22% and 10.5% peak prevalence for HPV 16 and 18 respectively among Australian women of age 30-39. The study of Newall and colleagues also reported high HPV prevalence in men age 50-59 for type 16 and 18 of HPV which this study revealed zero prevalence for. Similarly, Tigglear *et al.* (2012) reported that HPV16 peaked at age 25-40 years.

The lowest but significant HPV IgG seroprevalence found in this study was in a 1.6-year-old male, who might have contracted it prenatally or horizontally. This raises the pertinent question of the ideal time for introduction of HPV vaccine in routine immunization schedule in Nigeria. It also suggests that the vaccination should not be limited to females only. Syrjanen & Puranen (2000) reported that the concordance of HPV types detected in babies and their mothers is 57-69%, indicating that children might acquire HPV from variety of other sources. Besides, this study revealed case of seropositive ELISA to both HPV16 and 18. This agrees with a

study carried out in Ibadan by Franceschi *et al.* (2012) who reported that a certain percentage of patients were infected with more than one HPV genotype.

Out of all the risk factors considered for HPV IgG, none was found to have positive linear association. Some researchers have found association between HPV and some risk factors like sexual intercourse, contaminated shared objects and blood (Bodaghi *et al.*, 2005; Syjanen *et al.*, 2007). Current or past cigarette smoking has also been associated with acquisition of HPV infection, progression to lesions or cancer in a few studies while most failed to support it (Wang *et al.*, 2003; Winer *et al.* 2003; Syjanen *et al.*, 2007). Relatively low-sample size, hospital-based nature of this research and limited funding are our limitations. A larger community-based research would be needed to understudy all subtypes of HPV and associated risk factors.

## CONCLUSION

Seroprevalence of HPV has been found in under 5 age group and both sexes were discovered to have the antibody to human papillomavirus. A high percentage of those positive are at their middle age group. There is need for further studies on the activities of HPV among preadolescents and infants in Nigeria. A national survey study to capture all age groups from birth is hereby recommended for possible consideration of vaccination against HPV at lower age group and inclusion in National Programme for Immunization.

## Declaration of Interest

The authors declare no financial and non-financial competing interests.

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